

1/10

Fig. 1

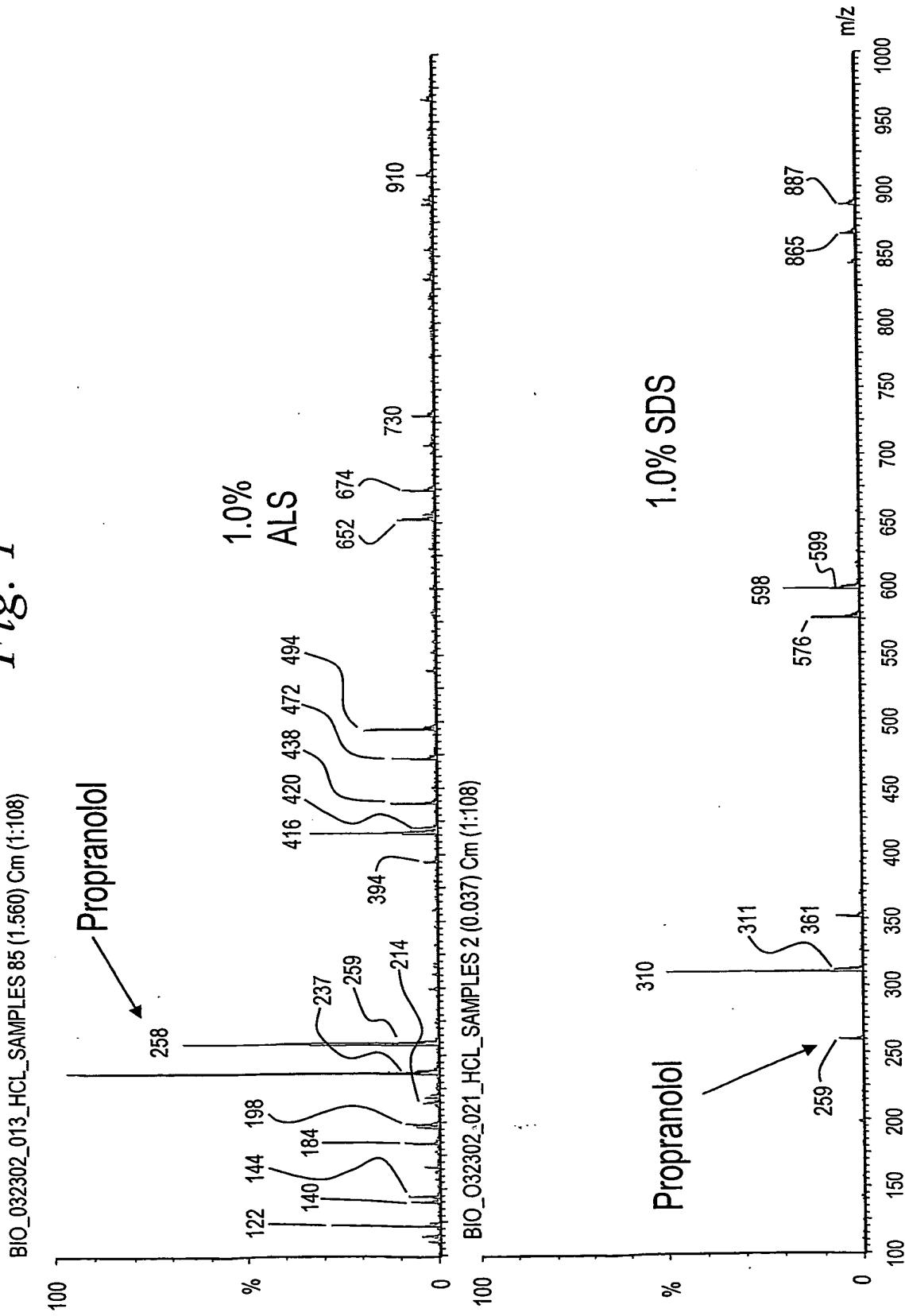
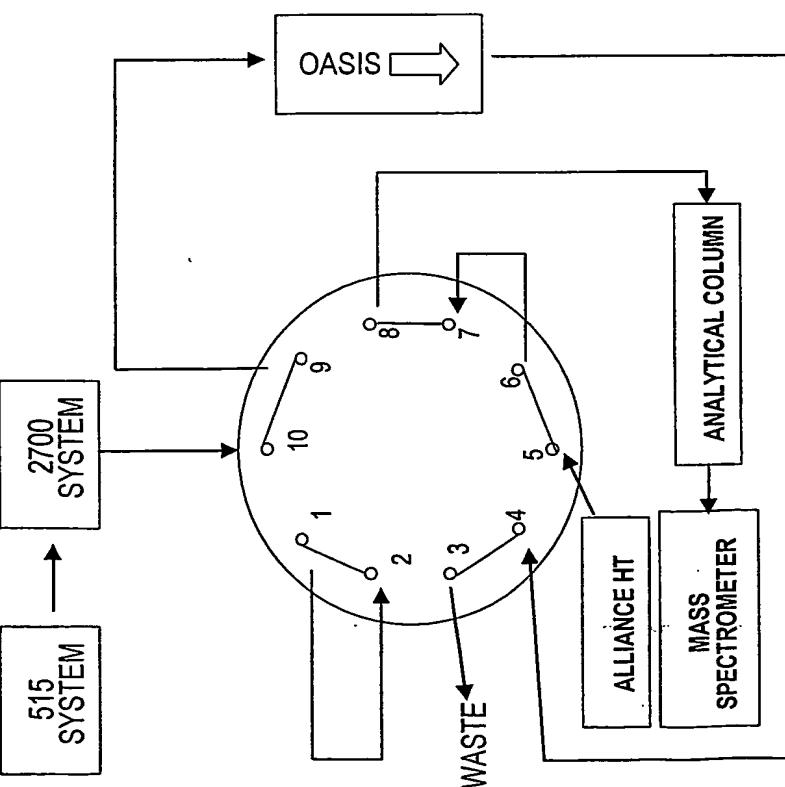
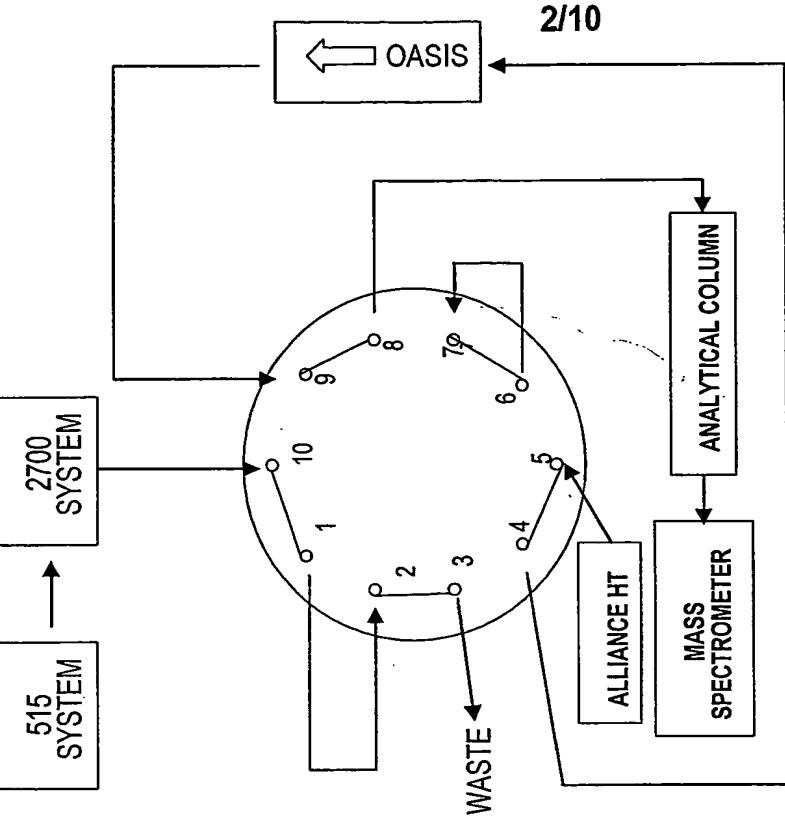


Fig. 2 Oasis® HLB/XTerra® Columns Configuration

Load position (backflush configuration)



Injection position (backflush configuration)



MS: Quattro Ultima Triple Quadrupole

Source: Electrospray positive

Source temperature: 150 °C

Desolvation gas: 600 L/hr

Gas cell: 2.0e-3 mbar

Cone Voltage: 20 volts

Collision energy: 20

LC₁: Alliance 2690 - 0.4 mL/min

LC₂: Waters 515 - 4.0 mL/min

Loading mobile phase: 100 % water

Eluting mobile phase: 1 minute gradient 5% ACN to 95% ACN

Eluting mobile phase additive: 0.5 % Formic acid

Extraction column temperature: room temperature

Switching valve: Rheodyne LabPro 10 ports, 2 position

10/516419

Fig. 3

HPLC Gradient and Wash Conditions

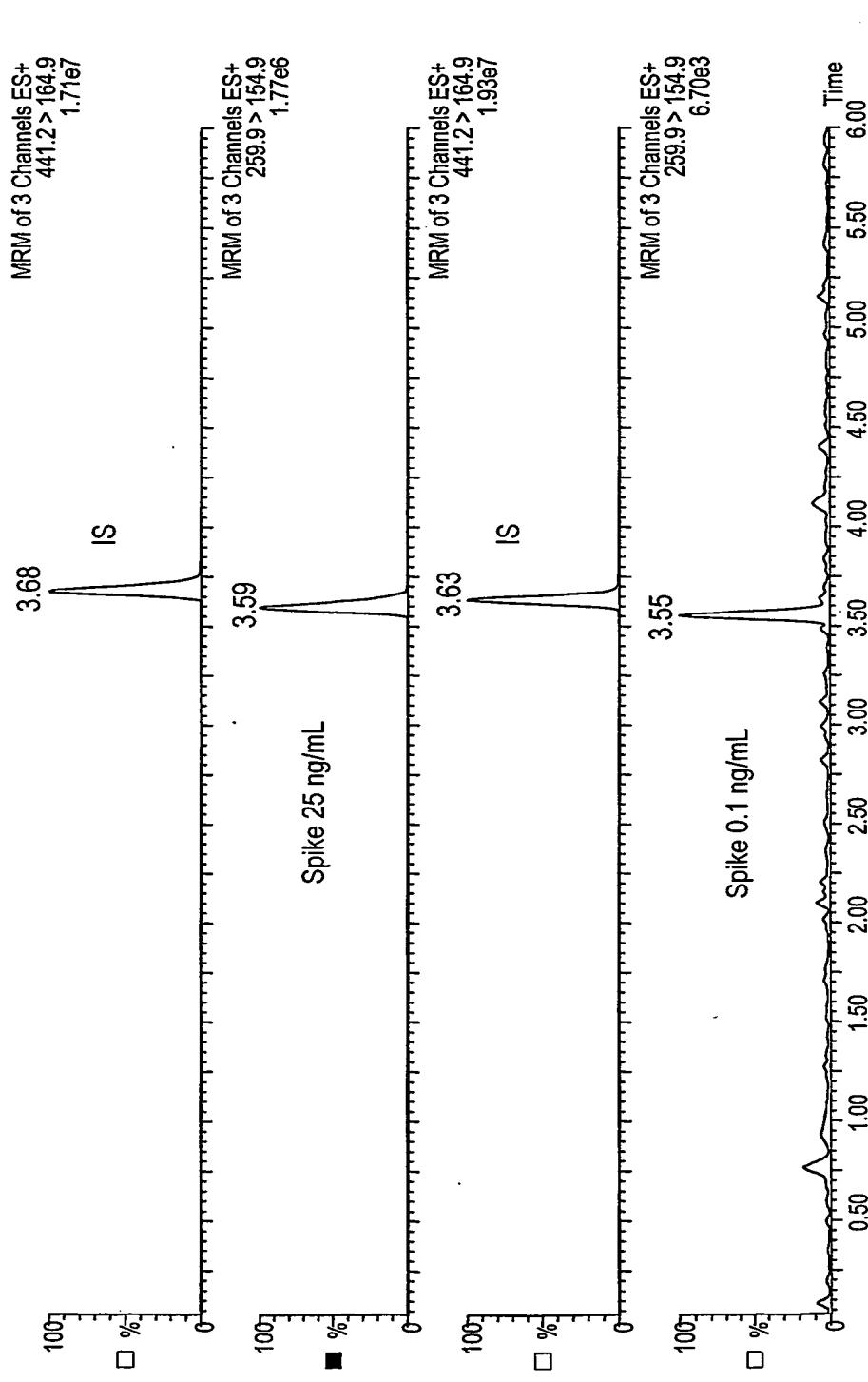
Time	HPLC gradient Flow 0.4 mL/min		Valve position	Function
	A	B		
0.0	5	95	position 1	Loading with 100 % H ₂ O
0.5	5	95	position 2	Elution with 1 min gradient
1.5	95	5		
4.40	95	5	position 1	Return to loading position
4.50	5	95		
6.0	5	95		

A - Acetonitrile + 0.5 % Formic Acid

B - Water + 0.5 % Formic Acid

Fig. 4

50/50 MeOH/ACN Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL



10/516419

5/10

Fig. 5 Ion Suppression of Surfactants

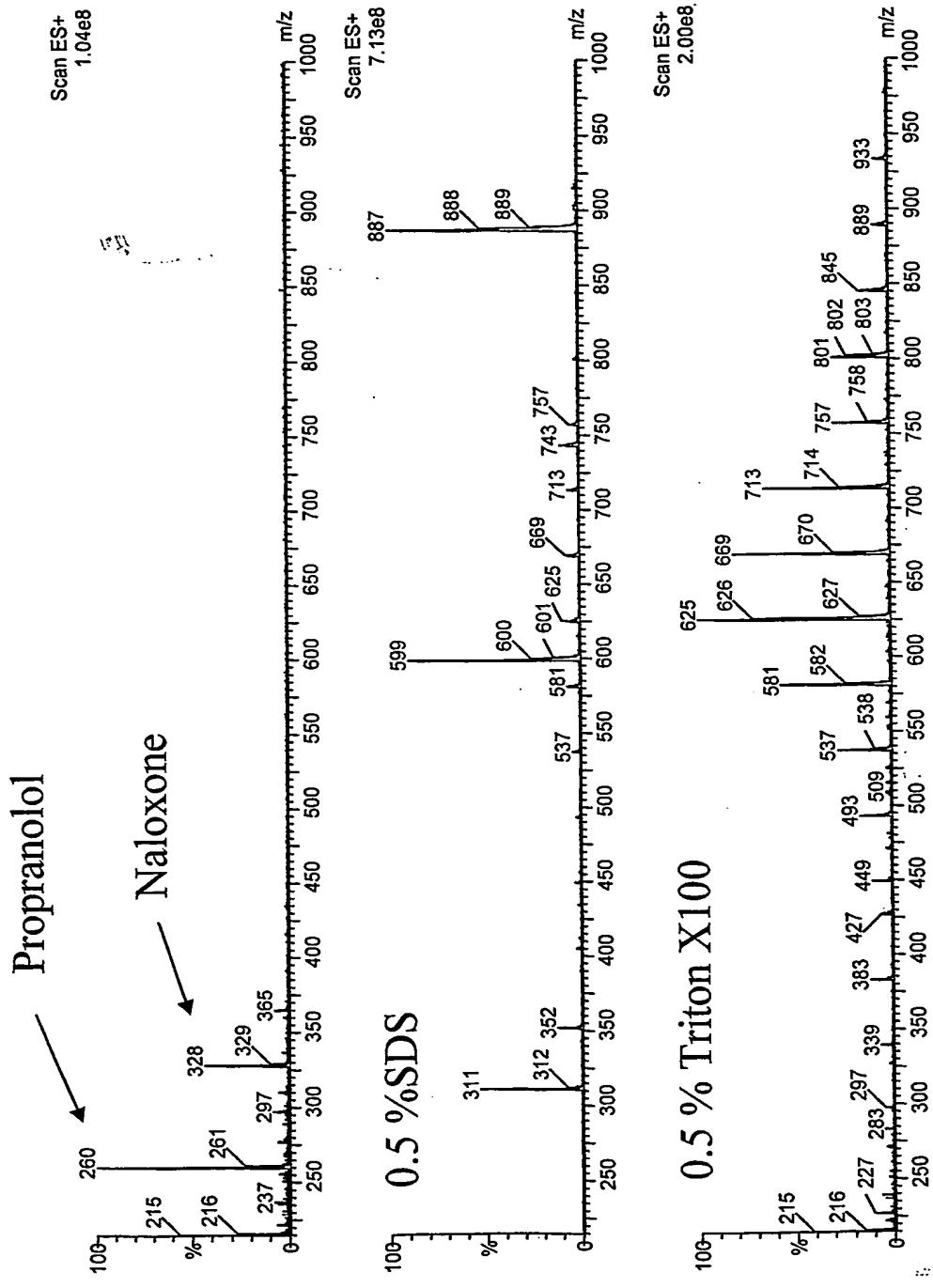
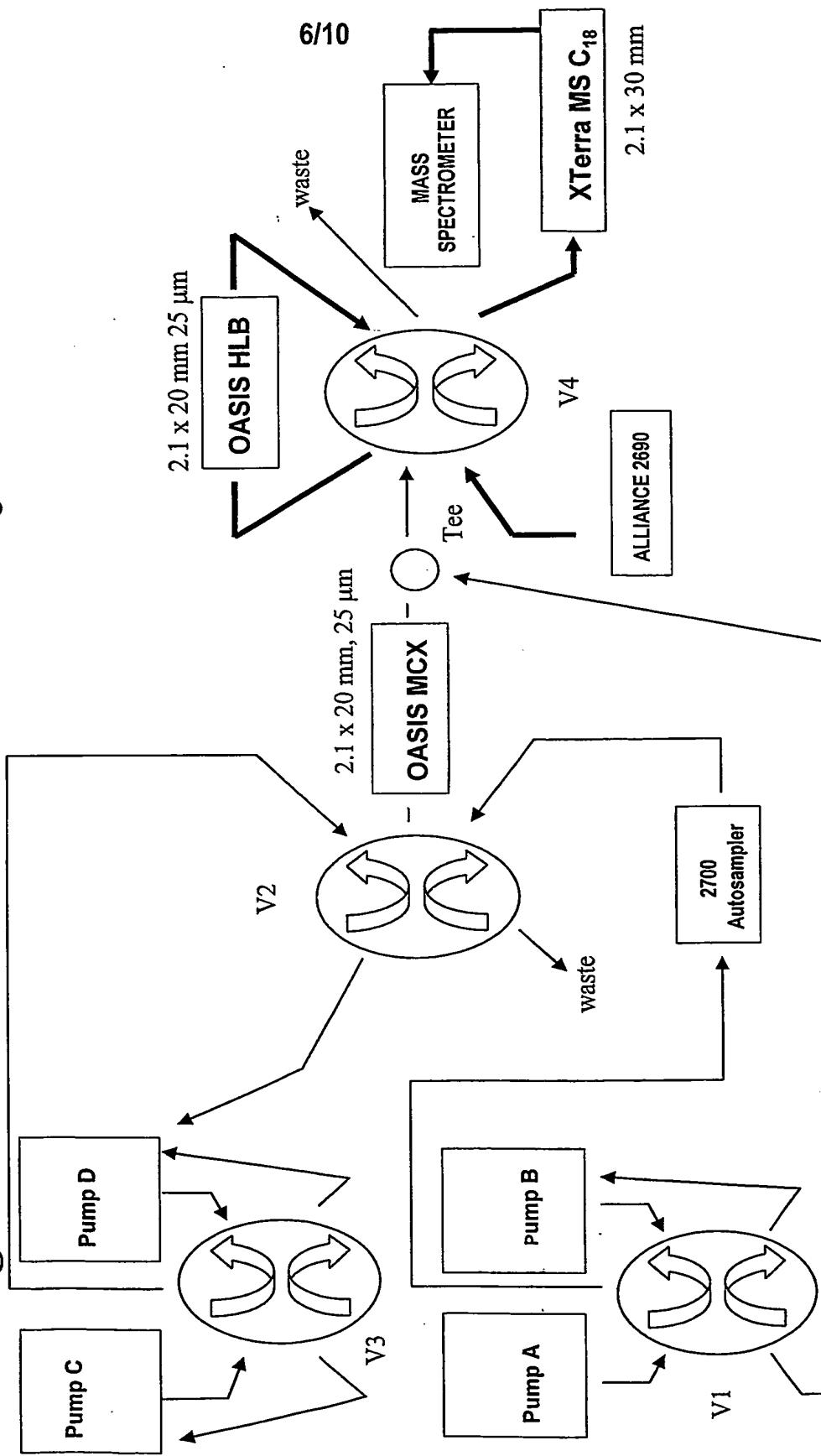


Fig. 6 Oasis® HLB / MCX / XTerra® Configuration



Pump A: 100 % water + 2 % NH₄OH
 Pump C: 100 % Water + 4 % Formic Acid

Pump B: 100 % Methanol
 Pump D: 100 % Methanol + 2 % NH₄OH

10/516419

7/10

Fig. 7 HPLC Gradient and Wash Conditions

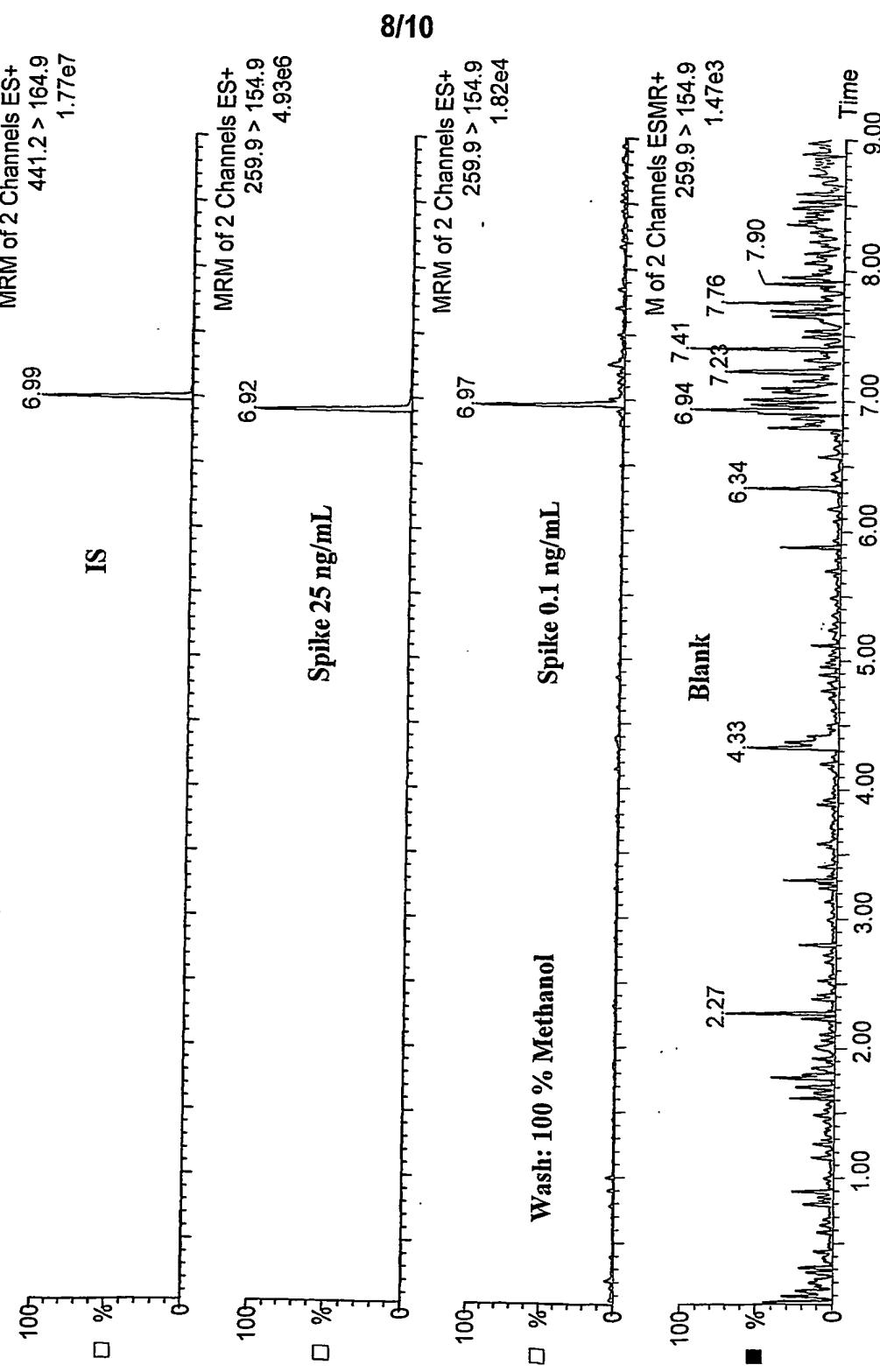
Time (min)	HPLC gradient Flow 0.4 mL/min		Valve Position V1, V2, V3, V4	Function
	A	B		
0.0	5	95	2-2-2-2	Load 100 % H ₂ O pH 11
1.0	5	95	1-1-2-2	Wash 100 % H ₂ O pH 2
2.0	5	95	1-2-2-2	Wash (see chromatograms)
3.0	5	95	1-1-1-2	Elution of MCX onto HLB (pH 11)
4.0	5	95	1-1-1-1	Elution of HLB onto Xterra (pH 3)
5.0	95	5		
7.0	95	5		
7.5	5	95		
8.0	5	95	2-2-2-2	Reset to starting position
9.0	5	95		

A - Acetonitrile + 0.5 % Formic Acid

B - Water + 0.5 % Formic Acid

10/516419***Fig. 8***

**1 % Triton X100 Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL**



10/516419**Fig. 9**

**1 % SDS Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL**

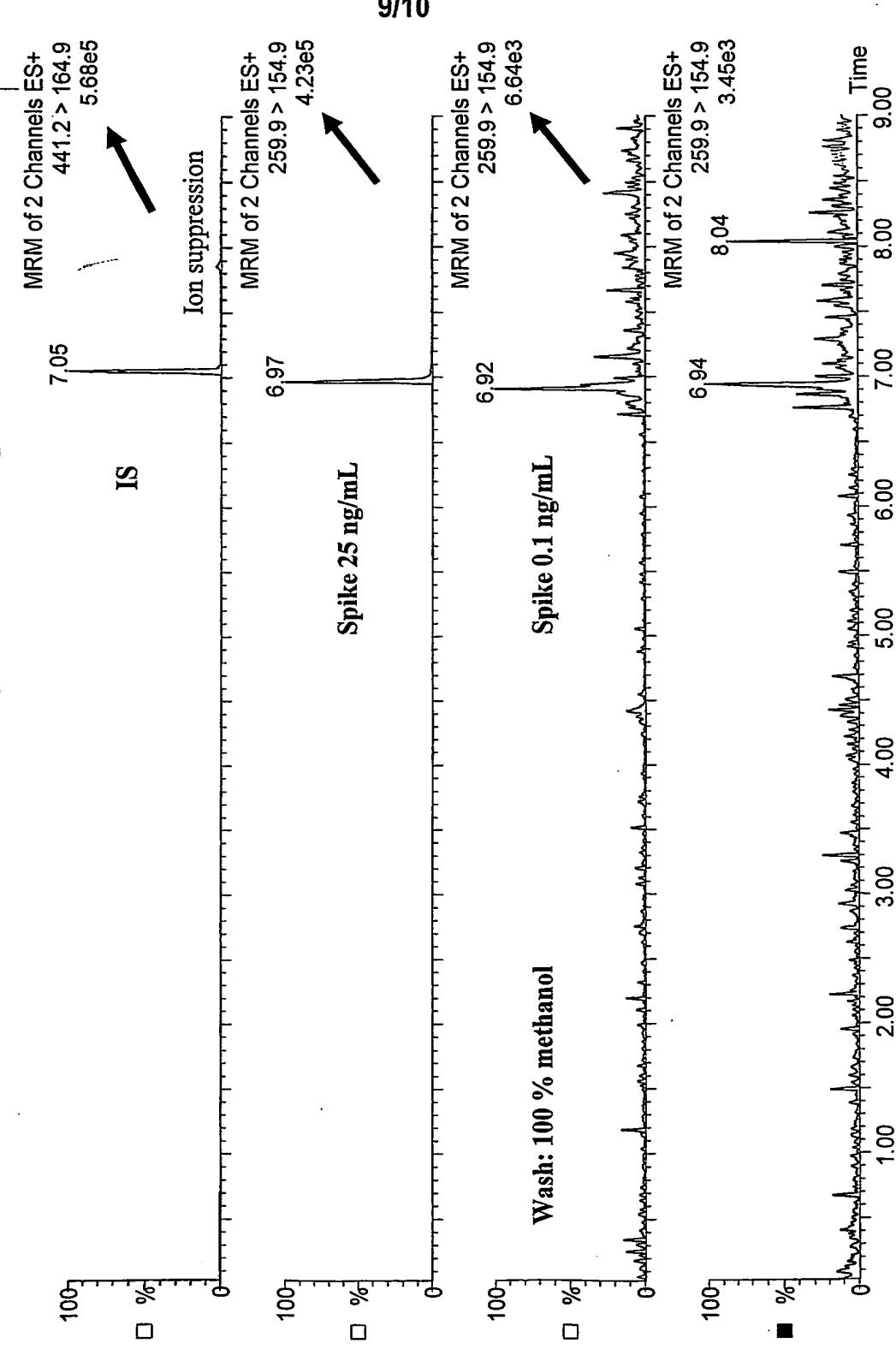


Fig. 10

**1 % SDS Cell Lysing
Propranolol at 0.1 ng/mL and 25 ng/mL**

